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CONFERENCE REPORT

Novel approaches to preclinical research and TB vaccine development

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SUMMARY

The 4th Global Forum on TB Vaccines, convened in Shanghai, China, from 21 – 24 April 2015, brought together a wide and diverse community involved in tuberculosis vaccine research and development to discuss the current status of, and future directions for this critical effort. This paper summarizes the sessions on Low-Dose NHP Challenge Models, Novel Approaches to Animal Models for TB Vaccine R&D, Novel Antigen Delivery Strategies, and Next Generation TB Vaccines and Vaccine Concepts. Summaries of all sessions from the 4th Global Forum are compiled in a special supplement of *Tuberculosis*. **[August 2016, Vol 99, Supp S1, S1–S30]**.

1. Introduction

Preclinical investigations, particularly challenge studies in small animal species such as mice and guinea pigs, often ultimately leading to challenge studies in non-human primates (NHPs), represent a critical step in the development of many vaccines. This is particularly true when developing vaccines for tuberculosis (TB) due to the lack in our understanding of the correlates of immunological protection against either TB disease, or infection with *Mycobacterium tuberculosis* (Mtb), which might otherwise guide the vaccine development process. Despite the importance of preclinical animal challenge models, however, a preclinical vaccine animal model known to reliably predict the potential for a vaccine to protect against TB in humans does not yet exist.

An additional challenge to TB vaccine development is the need to diversify the pipeline of vaccine candidates and vaccine strategies. Three vaccine delivery platforms – whole cell vaccines, adjuvanted protein subunits and subunit vaccines expressed in viral vectors – comprise the current clinical pipeline. All vaccines currently in clinical trials were selected based on their ability to induce classical TH-1 biased $CD4^+$ T-cell and, to a lesser extent, $CD8^+$ T-cell responses. Generating a broader diversity of immune responses is now considered a priority in assembling the components of a successful TB vaccine strategy.

2. Existing and novel approaches to preclinical assessment of new TB vaccines

Historically, with the NHP TB platforms, relevant biological effects of vaccine candidates (e.g., reduced pathology, improved clinical signs, or reduction in bacterial burden) have been assessed following high doses of challenge organisms, ranging from hundreds to more than a thousand colony forming units (CFUs) of virulent Mtb strains often delivered directly into the pulmonary space via bronchoscopic administration. Recently, the field has recognized that these supraphysiological challenge doses and the unnatural route of exposure may be misleading when assessing vaccine effects. Accordingly, recent efforts have focused on developing the NHP challenge model to better approximate the conditions of natural human Mtb infection.

Dr. Philana Ling Lin (University of Pittsburgh, USA) provided a comprehensive overview of cutting edge practices in NHP studies, focusing upon the application of modern technologies, such as positron emission tomography/computerized tomography (PET/CT) imaging and methods to assess the establishment of Mtb infection and subsequent disease progression following low-dose Mtb exposure. She also addressed the potential of using imaging to predict outcome of TB vaccines as well as the challenges to how this may translate to human infection. Unlike

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rhesus macaques (RM), which uniformly develop fulminant TB and die, generally within four months of a pulmonary Mtb challenge, 50% of cynomolgous macaques (CM) infected via bronchoscopic instillation with low dose (~25 CFU) Erdman strain Mtb develop asymptomatic, latent TB infection (LTBI), while the other 50% progress rapidly to active TB disease. This represents a reasonable approximation of Mtb infection in humans, where 90% of infected persons develop asymptomatic, latent infection while the other 10% develop active TB disease at some point in their lives. Despite normal-appearing chest X-rays, necropsies of these animals reveal a full spectrum of granuloma types, including caseous, non-necrotizing and fibrotic granulomas. In her experiments, PET/CT images were obtained at weeks 3, 6 and 8 after challenge and monthly thereafter. As early as three weeks after infection, animals that would later develop LTBI had fewer granulomas detected by PET/CT compared to active TB animals. On an individual granuloma level, granulomas from animals that would develop active disease had greater fluorodeoxyglucose (FDG) avidity by 6 weeks post-infection (termed "PET-hot" lesions) compared to those that would be latent. At 6 weeks, when pulmonary PET-hot lesions represent the development of secondary granulomas, CMs that developed LTBI demonstrated far fewer lesions compared to progressors, likely reflecting development of an adaptive immune response more capable of controlling Mtb infection as compared to CMs that progressed to disease [1]. Data so far suggest that greater pulmonary inflammation in the early stages of Mtb infection may indicate a poorer chance of controlling the infection at later stages. Increased lung inflammation early during infection was associated with increased mycobacterial burden detected at necropsy, 21 weeks after challenge. A reduction in the number of PET-hot lesions that develop between 3 and 6 weeks could represent an early indicator of vaccine effect, as, according to Lin, this is a critical period when a vaccine has an opportunity to increase the potential for a favorable outcome, such as decreasing the likelihood of progression to disease, or even preventing the establishment of Mtb infection. Lin concluded by noting that expanded utilization and harmonization of this important imaging tool would permit a more efficient and accurate assessment of CMs undergoing Mtb challenge following administration with a vaccine candidate and thereby accelerate and improve preclinical TB vaccine development.

Pia Steigler (University of Otago, New Zealand), addressed the question whether BCG-induced protection against TB is mediated by memory T-cells. The current dogma implies that TB vaccine-mediated protection largely is based on memory $CD4^+$ T-cells. Hence, the potential for vaccines to provide protective efficacy in (pre)clinical trials is evaluated by their ability to induce antigen-specific $CD4^+$ T-cell responses. However, mouse models illustrating the importance of memory $CD4^+$ T-cells may be flawed [2].

Steigler used a novel murine memory T-cell depletion model to investigate the role of BCG-induced memory $CD4^+$ and $CD8^+$ T-cells in protection against mycobacterial challenge. BCG-vaccinated mice lacking memory $CD4^+$, $CD8^+$ or both T-cell subsets were protected against mycobacterial challenge compared to unvaccinated mice. Her findings suggest that BCG-mediated protection can be independent of memory $CD4^+$ and $CD8^+$ T-cells.

Dr. Frank Verreck (Biomedical Primate Research Centre, the Netherlands) assessed both the variable response to Mtb challenge seen in different RM populations, and the effects of mucosal as compared to standard intradermal BCG administration in RM challenge models. Verreck noted that Indian RMs are more susceptible to TB disease than Chinese RMs following Mtb challenge. The efficacy of BCG-induced protection varies, but is independent of genotype. He cautioned that challenge experiments assessing vaccine-induced protection in NHPs needed to be assessed in the context of the natural susceptibility of each NHP genotype or cohort to Mtb infection. Additionally, Verreck noted that a mucosal BCG vaccination route (intra-nasal administration in his laboratory), demonstrates protective effects in RMs where standard intradermal BCG administration fails to protect, suggesting that administering TB vaccines via the respiratory mucosa should be explored to a greater extent.

Dr. Sally Sharpe (Public Health England, UK) updated her efforts to develop an ultra-low dose aerosol NHP challenge model. As discussed above, most NHP challenge models have used an Mtb dose that is far greater than the 1–5 CFU representing natural human Mtb exposure. Sharpe and colleagues have developed an ultra-low dose challenge model in RMs and CMs in which approximately 25 CFUs are presented via aerosol with an estimated retained dose of 3–4 CFUs, inducing as few as five primary lesions in the lung. CT scans collected 3 weeks after aerosol exposure demonstrated that the number of lesions was similarly low and consistent across species (range: 1–10) with medians of six nodules for RMs and four nodules in CMs. Using this ultra-low dose, Erdman strain aerosol Mtb challenge, they found that RMs developed slow but progressive TB disease. In contrast, CMs proved more capable of controlling Mtb infection without inevitable progression to disease, as also found by Lin and colleagues (discussed above). Sharpe concluded by noting the importance of further developing this ultra-low challenge model given its close approximation to natural human Mtb exposure and the potential for providing preclinical TB vaccine efficacy assessments more reflective of the clinical outcome in humans [3].

Dr. Angelo Izzo (Colorado State University, USA) described the potential utility of the guinea pig (GP) Mtb challenge model when assessing candidate TB vaccines. The Hartley strain of GP is highly susceptible to TB, and BCG vaccination prolongs survival of Hartley GPs following Mtb challenge. Izzo described three general categories of disease progression manifest in Mtb-infected GPs: acute, progressive and chronic. Izzo and colleagues studied the relationship between selected immunological and non-immunological factors and the extent of post-Mtb challenge survival. In both unvaccinated GP and BCG vaccinated GP groups, some survived nearly two years post infection, suggesting that survivability post-Mtb challenge was not necessarily conferred by BCG vaccination but was likely to have resulted from immune responses innate to particular GPs. He noted that a greater elucidation of the natural history of Mtb infection in GPs is needed to permit a better understanding of the functional impact of vaccination on altering the natural course of Mtb infection in GPs.

3. Novel Antigen Delivery Strategies

The development of new viral vectors for Mtb vaccines, including Sendai virus, lentivirus, parainfluenza virus 2, and influenza virus, provided the focus for a breakout session on Novel Antigen Delivery Strategies. Viral vector vaccines generally consist of a live, attenuated virus genetically engineered to deliver a transgene encoding a foreign antigen(s) from an unrelated organism. Similar to whole cell vaccines, viral vectors have the potential

to engage the innate immune system through activation of pattern recognition receptors (e.g., Toll-like receptors) and drive the production of antigen *in vivo*.

Dr. Zhidong Hu (Shanghai Public Health Clinical Center, Fudan University, China) described the use of a Sendai virus (SeV) vectored TB vaccine developed as a replication-deficient carrier for Ag85A and Ag85B antigens (SeV85AB). According to Dr. Hu, a single intranasal administration in mice induced potent, antigen-specific T-cell responses in the draining lymph nodes and lung, to the same extent as BCG. Following SeV85AB vaccination, they observed a greater reduction in Mtb load and an enhanced pulmonary $CD8^+$ T-cell response as compared to Mtb challenge in BCG-vaccinated mice. Hu suggested that the SeV85AB vaccine may represent a potent booster to BCG, given that the SeV85AB vaccine demonstrated a 0.5 log improvement in protection in a mouse challenge when used as a boost to BCG, as compared to a degree of protection similar to that of BCG when used as a stand-alone vaccine. Additionally, by inducing high levels of local and systemic $CD8^+$ T-cell responses, SeV85AB may represent a promising therapeutic vaccine candidate for use in persons with active TB disease. When questioned about the safety of an SeV-vectored construct for human use, Hu noted that demonstration of safety would be a particularly important component of any effort to move this construct forward in clinical trials.

Dr. Mohamad F Jamiluddin (Theravectys, France) described a therapeutic vaccine using optimized lentiviral vectors (LVs) partially derived from HIV for potential use in treating persons with multidrug-resistant TB (MDR-TB). A specific modification to the virus provided the LV construct with a unique ability to express the transgene efficiently in non-dividing cells, including dendritic cells [4]. The packaging capacity of the LV system is relatively large, allowing up to 15 Kb insert to be included and therefore offering the advantage of generating a component LV vector vaccine containing genes expressing antigens found during different stages of Mtb infection. Four different LV constructs were designed, each encompassing classical, latent and resuscitation antigens from Mtb. These constructs will be further evaluated in mouse protection studies.

Prof. Dr. Yasuhiro Yasutomi (National Institutes of Biomedical Innovation, Health and Nutrition, Japan) presented the development of a recombinant human parainfluenza type 2 virus (rHPIV2) vectored intranasal vaccine against Mtb infection. The deletion of the M gene results in a single cycle infection. Further attenuation was attained by eliminating selected HPIV genes that are involved in immune evasion. An HPIV2 vector expressing Ag85B was effective in inducing high numbers of IFN-γ secreting cells in lymph nodes and locally in bronchoalveolar lavage (BAL) cells after intranasal administration in mice. After Mtb challenge, antigen specific systemic and mucosal responses were detected that were stronger than those observed in subcutaneous BCG immunized mice. The mechanism is yet to be understood since mice are not susceptible to HPIV2 infection. Additionally, the HPIV2 viral vectors offer potent adjuvant activity by activation of innate immune receptors through viral ssRNA. The rHPIV2 vectored vaccine was shown to be a single cycle vector, i.e., one that does not demonstrate multiple-stage growth in cells, and may therefore considered to be safer than vectors capable of extended replication. This was only confirmed in M-gene transfected cell lines, however, and questions regarding *in vivo* safety remain to be addressed.

Dr. Marina Stukova (Research Institute of Influenza, Russia) presented a phase 1 trial with an influenza vector-based TB vaccine, expressing Ag85A and ESAT-6 (TB/FLU-04L). Dr. Stukova noted that live, influenza virus, attenuated through truncation of the viral NS1 protein, has a benign safety profile in humans and is highly immunogenic, making it an attractive candidate to serve as a vaccine vector. A genetically stable, replication-deficient influenza viral vector harboring ESAT6-Ag85A was generated which showed increased antigen-specific IFN-γ responses in mice and cynomolgus monkeys. When tested in a clinical phase 1 study as a nasal spray, the vaccine was well tolerated with no serious adverse events or viral shedding reported. Local nasal cytokine production (IL-1β, TNFα and IL2) was determined as early immune responses to vaccination. Both antigen-specific memory $CD4^+$ and $CD8^+$ T-cell responses were observed after two vaccinations. T-cell responses to Ag85A peaked at 21 days and subsequently decreased, while responses to ESAT-6 peaked at 21 days but were sustained throughout the course of study. No antibody response against influenza vector was noted.

Prof. Warwick Britton (University of Sydney, Australia) presented preliminary data from studies of mice immunized intranasally with a recombinant influenza A virus-based vaccine (rIAV). The PR8 H1N1 and X31 H3N2 strains of IAV were genetically modified to express specific mycobacterial antigens, including Ag85B and TB10.4. These constructs induced strong, antigen-specific $CD4^+$ T-cell responses in the lung that persisted over 12 weeks post infection. A single administration of the PR8 virus expressing one Mtb-specific $CD4^+$ T-cell epitope from Ag85B, but not an Mtb-specific $CD8^+$ T-cell epitope from TB10.4, was sufficient to confer protection in the lungs against Mtb challenge, with efficacy similar to that of BCG. Heterologous virus boosting resulted in significantly higher numbers of IFN-γ producing $CD4^+$ T-cells compared to immunization with single viruses. Sequential immunization with PR8-p25 and X31-p25 strongly boosted T-cell responses after BCG prime. Prior BCG immunization boosted with PR8-p25 and X31-p25 enhanced these pulmonary $CD4^+$ T-cell responses against Mtb even more efficiently. Therefore rIAV vectors can stimulate strong protective $CD4^+$ T cell responses against Mtb [5].

4. Next generation TB vaccines and new vaccine concepts

Dr. Olivier Neyrolles, (IPBS, CNRS-University of Toulouse, France) introduced the objectives of a new TB vaccine research consortium, TBVAC2020. The consortium is supported by Horizon 2020, the European Union's current Framework Programme for Research and Innovation, and dedicates a major part of the work program to discovery of new antigens, delivery systems, immunization strategies, and novel live vaccines. TBVAC2020 is coordinated through the TuBerculosis Vaccine Initiative (TBVI) and works with the 40 consortium members of TBVAC2020. The main aim of this program is to broaden and deepen the diversity of the preclinical vaccine pipeline. The consortium operates a portfolio management approach built around internationally agreed gating criteria for product development in a series of well-defined animal models.

TBVAC2020 will be focused on the following areas: diversity of antigens & delivery systems; diversity of preclinical models; head-to-head testing in animal models & preclinical development; clinical development; diversity of biomarkers; and portfolio management.

TBVAC2020 will attempt to discover novel antigens and epitopes using innovative technologies, explore novel stage-specific antigens using candidate-based and genome-wide expression

profiling, evaluate and exploit antibody-mediated protection using patient-derived antibodies and undertake Mtb mutant library screening, discover and exploit novel lipid and glycoconjugate antigens in vitro and *in vivo* using innovative tools (e.g. recombinant CD1b, CD1b-Tg mice), and evaluate viral vector-based liposomes and nanoparticle-based technologies as vaccine candidates. To select better candidates, they will use a portfolio management committee (PMC); preclinical models that resemble infection in humans; independent head-to-head animal testing; and independent Phase 1 clinical testing.

Dr. Sarah Marcus (University of Wisconsin–Madison, USA) presented ongoing work to develop live, attenuated Mtb-based vaccines and assess the potential of these vaccines to provide superior protection against TB as compared to BCG vaccine given their derivation from pathogenic Mtb and potentially prolonged persistence in the host compared to BCG [6]. She described two attenuated Mtb mutants, involving the deletions *ΔmosR* and *ΔechA7*. The mosR and ech7 gene regions are necessary for virulence; the MosR region includes 163 negatively regulated genes [7]. Both strains were tested as vaccines in C57BL/6 mice compared to BCG and a PBS control, injecting 10^6 CFU of the vaccine strains subcutaneously 8 weeks prior to aerosol challenge with ~40 CFU of an Mtb Beijing strain. Both vaccine strains persisted up to 16 weeks in the spleen and lungs of vaccinated mice. BCG, in contrast, was not detected after administration. The bacterial burden data showed nearly a log reduction in the lungs of either *ΔmosR* or *ΔechA7* vaccinated mice compared to BCG vaccinated mice, and a 2 log reduction in viable bacterial load compared to PBS control mice at both days 30 and 60 post-challenge. Remarkably, by day 60 post-challenge, no Mtb colonization was observed in the lungs of *ΔmosR* vaccinated mice. Flow cytometry demonstrated that vaccination with *ΔechA7* primed an Mtb specific IFN-gamma response from $CD4^+$ T-cells isolated from the lungs 8 weeks following vaccination, significantly greater than that from BCG vaccinated mice, which showed no response. Cells from the lungs of *ΔmosR* vaccinated mice also showed an enhanced response. Additionally, ELISA assays at 60 days post challenge demonstrated significantly higher Mtb specific IFN-gamma production by splenocytes isolated from mice vaccinated with *ΔmosR* compared to those vaccinated with BCG. These data suggest that the live attenuated vaccines tested in this study demonstrated better protection than BCG. Future plans include the placement of both *mosR* and *echA7* deletions in the same Mtb organism and subsequent assessment in Mtb challenge experiments.

5. Conclusion

Efforts to develop better animal challenge models of TB infection are ongoing and represent a critically important effort in accelerating TB vaccine development. Developing a better understanding of the implications of post-vaccine Mtb challenge in small animal models such as mice and guinea pigs, and applying new imaging technology such as the PET/CT scan and ultra low doses Mtb administration to NHP challenge models, are high priority strategies that offer more rapid and accurate assessments of potential vaccine efficacy in humans. Additionally, the need to obtain a broader view of the diversity of immune response induced by TB vaccines via the development of novel vaccine delivery strategies, including viral vector platforms, has been recognized as a high priority in the field and represents an area of intense and promising activity. The ongoing support for these closely related preclinical strategies will be critical to ensure that robust and efficiently assessed pipeline array of vaccine concepts are available for future advancement into clinical testing.

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